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Morphological and molecular evidence reveal a new species of the genus *Paramesotriton* (Caudata, Salamandridae) from Chongqing, China

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Abstract

Based on morphological and molecular phylogenetic evidence, this study describes a new species of the salamandrid genus *Paramesotriton* discovered in Pengshui Miao and Tujia Autonomous County, Chongqing Municipality, China. The taxonomic status of this new species was established through integrated morphological characterization and multi-gene phylogenetic analysis (mitochondrial genes *ND2* and *Cytb*, and nuclear gene *POMC*). The new species is primarily diagnosed by the following characteristics: presence of horn-like projections posterior to the head; black ground coloration on the throat and venter adorned with irregular orange-red blotches; bluntly rounded tail tip; and overlapping digits when the limbs are pressed along the body flank with the forelimb backward and the hind limb forward. Additional diagnostic characteristics include the absence of vestigial gills and gill filaments and lack of yellow spots on lateral sides of dorsum and tail. Molecular phylogenetic analyses place the new species within the *P. caudopunctatus* group (Clade B), forming a strongly supported sister group (BI = 0.91, ML = 74) to the combined clade comprising *P. maolanensis*, *P. wumengshanensis* and *P. longliensis*. The minimum genetic distance between the new species and its closest relative *P. maolanensis* reaches 0.78% (*Cytb*, p-distances), exceeding interspecific divergence observed among some congeners (e.g., 0.63% between *P. maolanensis* and *P. longliensis*), further supporting its distinct species status. This discovery further validates the persistence of cryptic diversity in the karst mountain regions of southwestern China, such as the Wuling Mountains. The new species has a restricted distribution and specialization to stream habitats at elevations of 700–1000 meters which render it is highly susceptible to threats from habitat fragmentation and illegal exploitation. It is recommended that this species should be categorized as "threatened" and that urgent conservation measures need to be implemented.

Key Words

cryptic species, *Paramesotriton chongqingensis* sp. nov., Southwestern China, Wuling Mountains

Introduction

The genus *Paramesotriton* Chang, 1935 (Caudata, Salamandridae) is an amphibian group endemic to East Asia. Currently comprising 15 recognized species, it is primarily distributed across southwestern to central-southern China as well as northern Vietnam (Raffaëlli 2022; Frost 2025). The genus exhibits a complex taxonomic history, having previously encompassed several synonymous genera (e.g., *Mesotriton* Bourret, 1934; *Trituroides* Chang, 1935).

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The phylogenetic position of *Paramesotriton* and interspecific relationships within the genus have been persistent research foci. Chan et al. (2001) proposed *Paramesotriton* as the sister group to *Pachytriton*. Subsequent molecular phylogenetic studies (Weisrock et al. 2006; Steinfartz et al. 2007; Zhang et al. 2008) revealed complex evolutionary relationships among *Paramesotriton*, *Pachytriton*, and *Cynops*, with some analyses supporting *Paramesotriton* and *Pachytriton* as a monophyletic group nested within a paraphyletic *Cynops*. Dubois and Raffaëlli (2009) advocated for the recognition of *Laotriton* as a distinct genus to render *Paramesotriton* monophyletic, and subdivided *Paramesotriton* into two subgenera: *Paramesotriton* and *Allomesotriton*. The work of Gu et al. (2012b) supported the monophyly of *Paramesotriton* and its sister relationship to *Laotriton*, while also confirming the monophyly of these two subgenera. Recent studies (Wu et al. 2009; Yuan et al. 2014; Luo et al. 2021, 2022; Yuan et al. 2022a), utilizing molecular data, have significantly advanced the taxonomic revision and understanding of species diversity within the genus by exploring intrageneric phylogenetic relationships, cryptic diversity, biogeographic history, and species delimitation. Notably, research by Luo et al. (2021, 2022) on the *P. caudopunctatus* group revealed novel cryptic lineages. This group is characterized by specialized adaptations to terrestrial and aquatic habitats, typically inhabiting montane streams and adjacent forests. However, habitat fragmentation and illegal collection pose significant threats to their survival (Sparreboom 2014).

Although recent integrative approaches combining morphological and molecular data (e.g., Yuan et al. 2014; Luo et al. 2021) have progressively refined the taxonomic framework of *Paramesotriton*, the complex topography and biodiversity hotspots of southwestern China, such as the Wuling Mountains, suggest that undiscovered cryptic species may still exist.

In this context, during a herpetological survey conducted in Ditang Township, Pengshui Miao and Tujia Autonomous County, Chongqing, China, we collected six specimens of unidentified *Paramesotriton*. Morphological and molecular analyses revealed significant differences from known congeners, leading us to describe these specimens as a new species.

Materials and methods

Sampling

During amphibian surveys conducted in Ditang Township, Pengshui Miao and Tujia Autonomous County, Chongqing Municipality, China, on 18 May 2023 and 30 June 2025, specimens were collected at night from the bottom of the stream (Fig. 1). Among them, six specimens of the salamandrid genus *Paramesotriton* were collected and photographed, with one individual obtained on 18 May 2023 and five on 30 June 2025, during night surveys from 19:00 to 02:00. After taking photographs, the specimens were euthanized using benzocaine. Specifically, they were immersed in a 0.1% benzocaine aqueous solution within a sealed wide-mouth bottle and observed until all voluntary movement ceased and no reflex response to a firm tail pinch could be elicited. The specimens were then removed and rinsed thoroughly with clean water prior to tissue sampling. Muscle tissue was taken from the hindlimb thigh muscle of each specimen and preserved in 95% ethanol for subsequent DNA extraction. Specimens were fixed in 10% formalin for 24 hours before long-term storage in 75% ethanol. The procedures for DNA tissue sampling and specimen fixation follow the protocols detailed by Chen et al. (2021). Voucher specimens were deposited at Chongqing Natural History Museum (CMNH), Chongqing, China, with voucher numbers: CMNH 1575,CMNH 1658,CMNH 1659,CMNH 1660,CMNH 1661,CMNH 1662. The protocols (No. JHDXKJLL2025-034) of the Animal Care and Ethics Committee were followed for the proper treatment of animals. This study was conducted with permissions and support from the Chongqing Municipal Forestry Bureau through the "Supplementary Survey of Nationally Protected Terrestrial Wild Animals in Chongqing" project (No. sxxycq-2021-086).

Morphological characters

Morphological characters were measured on the voucher specimens. Measurements were recorded to the nearest 0.1 mm with digital calipers by Qi Ma following Fei et al. (2006). The measurements taken were as follows: TOL (total length); SVL (snout-vent length); TAL (tail length); HL (head length); HW (head width); TRL (trunk length); SL (snout length); ED (eye diameter); IOD (interorbital distance); EN (eye-nostril distance); IND (internarial distance); AG (axilla-groin length); FLL (forelimb length); HLL (hindlimb length); TAD (maximum tail depth); CHW (chest width); TAW (tail width). Comparative morphological data for recognized species of *Paramesotriton* were taken from Fei et al. (2006), Fei and Ye (2016), Li et al. (2008a, 2008b), Zhao et al. (2008), Wu et al. (2009), Gu et al. (2012a, 2012b), Wang et al. (2013), Yuan et al. (2014, 2016), and Luo et al. (2025).

DNA extraction, PCR and sequencing

Genomic DNA was extracted, using a DNA extraction kit from Tiangen Biotech (Beijing) Co., Ltd. For phylogenetic analysis, two mitochondrial genes, NADH dehydrogenase subunit 2 (*ND2*) and Cytochrome b (*Cytb*), as well as one nuclear gene, proopiomelanocortin (*POMC*), were amplified. The primers used for *ND2* were 3787F

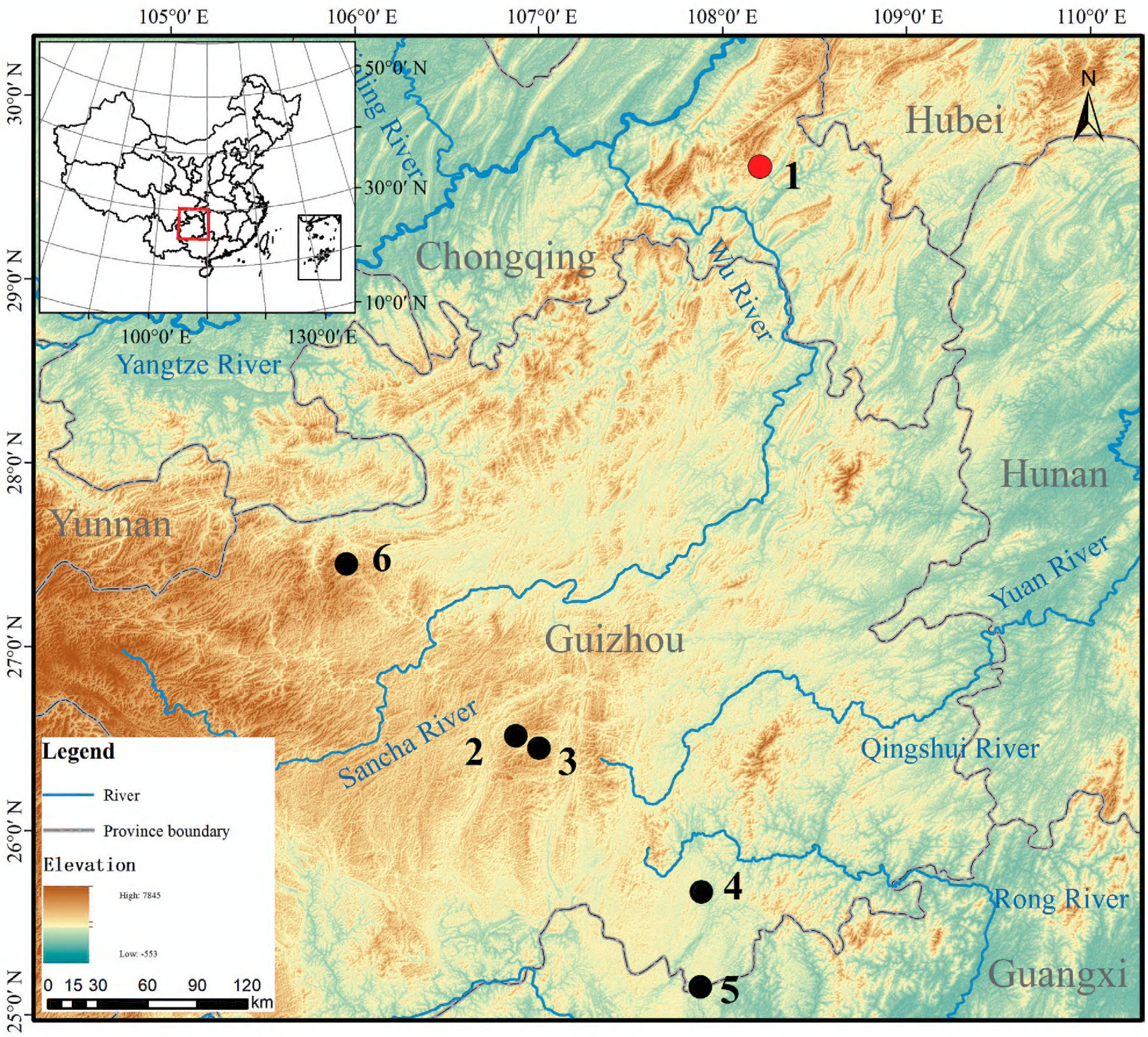

Figure 1. Distribution of the new species and its sister taxon. An inset map in the upper left corner shows the geographic context, with a red box indicating the area of the main map. 1: *Paramesotriton chongqingensis* sp. nov.; 2–3: *P. longliensis*; 4–5: *P. maolanensis*; 6 *P. wumengshanensis*. The elevational background data was derived from the GDEMV2 30-meter resolution digital elevation model, obtained from the Geospatial Data Cloud website (https://www.gscloud.cn). (Distribution of *P. longliensis* refers to Li et al. 2008b; Yuan et al. 2022a; Distribution of *P. maolanensis* refers to Gu et al. 2012a; Yuan et al. 2022a; Distribution of *P. wumengshanensis* refers to Luo et al. 2025).

(5’-TCGTGCGCCCACTACACTAT-3’) and 5081R (5’-GTCGTAGGGTCAAAGCCTGC-3’), for *Cytb* were 14052F (5’-CCTGGGCTCTAACCAAGACC-3’) and 15293R (5’-TCGGCTTACAAGACCGATGT-3’), and for *POMC* were DRV F3 (5’-ATGAGCCAYTTYCGCTGGAA-3’) and DRV R1 (5’-GGCRTTYTTGAAWAGAGTCATTAGWGG-3’) (Wu et al. 2010; Gu et al. 2012a). PCR amplification was performed under the following cycling conditions: an initial denaturation step at 94 °C for 2 minutes (for *ND2* and *Cytb*) or 5 minutes (for *POMC*), followed by 35 cycles of denaturation at 94 °C for 30 seconds (*ND2* and *Cytb*) or 45 seconds (*POMC*), annealing at 52 °C for 45 seconds (*ND2* and *Cytb*) or 48 °C for 30 seconds (*POMC*), extension at 72 °C for 90 seconds (*ND2* and *Cytb*) or 1 minute (*POMC*), and a final extension at 72 °C for 10 minutes. The PCR products were detected by 1% agarose gel electrophoresis and then sent to Shengong Biotechnology (Shanghai) Co., Ltd. for sequencing. All sequences were assembled from forward and reverse reads and manually edited using AutoSeqMan (Sun 2018). The new sequences have been deposited in the GenBase database (Bu et al. 2024) of the National Genomics Data Center (CNCB-NGDC Members and Partners 2024) at the Beijing Institute of Genomics, Chinese Academy of Sciences / China National Center for Bioinformation, and are publicly accessible at https://ngdc.cncb.ac.cn/genbase (Suppl. material 1).

Phylogenetic analyses

A total of 32 *ND2*, 32 *Cytb*, and 26 *POMC* gene sequences were retrieved from GenBank for phylogenetic analysis. *Euproctus platycephalus* (Gravenhorst, 1829) and *Leptomantis gauni* (Inger, 1966) were included in the data as outgroups. In PhyloSuite v1.2.3 (Zhang et al. 2020), based on the combined sequences of *ND2*, *Cytb* and *POMC* gene, we constructed a Bayesian phylogenies using MRBAYES v3.2.7a (Ronquist et al. 2012) and a Maximum likelihood phylogenies using IQ-TREE v2.2.0 (Nguyen et al. 2015). Maximum likelihood (ML) phylogenies were inferred under the TIM+F+I+G4 model selected by ModelFinder v2.2.0 (Kalyaanamoorthy et al. 2017) according to AIC criterion, with 50000 ultrafast bootstrap (UFB) replicates (Hoang et al. 2018) until a correlation coefficient of of at least 0.99 was achieved. Bayesian Inference (BI) phylogenies were inferred under HKY+F+I+G4 model selected by ModelFinder v2.2.0 (Kalyaanamoorthy et al. 2017) according to BIC criterion. Each BI analysis was run independently using four Markov Chain Monte Carlo chains (three heated chains and one cold chain) starting with a random tree; each chain was run for 2×10^7 generations and sampled every 1000 generations. Convergence of the data runs was confirmed when the average standard deviation of split frequencies was less than 0.01. Additionally, uncorrected p-distance of *ND2*, *Cytb* and *POMC* gene were conducted in MEGA v.11.0.13 (Tamura et al. 2021).

Results

Molecular phylogenetics

Based on the concatenated sequence matrix of mitochondrial genes (*ND2*, *Cytb*) and the nuclear gene (*POMC*), comprising 38 individuals with a total length of 2549 base pairs, including 912 variable sites and 696 parsimony-informative sites (including outgroups).

Both Maximum Likelihood (ML) and Bayesian Inference (BI) analyses generated nearly identical phylogenies (Fig. 2). Most terminal nodes exhibited relatively high support values. The monophyly of *Paramesotriton* was strongly supported (BPP = 1, BS = 100), forming two major clades designated as Clade A and Clade B, corresponding respectively to the *P. chinensis* group and the *P. caudopunctatus* group. Clade A comprised *P. auranti-us*, *P. chinensis*, *P. deloustali*, *P. fuzhongensis*, *P. guangx-iensis*, *P. hongkongensis*, *P. labiatus*, *P. qixilingensis*, and *P. yunwuensis*, whereas Clade B included *P. cau-dopunctatus*, *P. wulingensis*, *P. zhijinensis*, *P. longliensis*, *P. wumengshanensis*, and *P. maolanensis*. Our sample was nested within Clade B (BPP = 1, BS = 100), forming a highly supported monophyletic group (BPP = 1, BS = 98), distinct from all recognized species lineages (e.g., *P. maolanensis*, *P. wumengshanensis* and*P. longliensis*). The novel lineage was strongly supported as sister to the clade comprising *P. maolanensis*, *P. wumengshanensis* and *P. longliensis* (BPP = 0.91, BS = 74), indicating its evolutionary independence. *P. zhijinensis* was positioned on an external branch of this cluster (Fig. 2).

The genetic distance within our samples was 0%, confirming a highly homogeneous genetic unit. The minimum genetic distance to its closest relative, *P. maolan-ensis*, reached 0.78% (based on *Cytb* p-distances, Suppl. material 2). This value substantially exceeds intraspecific variations observed in several congeners (e.g., 0% in *P. maolanensis*, 0.16% in *P. longliensis*, 0% in *P. zhijin-ensis*) and surpasses the interspecific distance of 0.63% between *P. maolanensis* and *P. longliensis*. Integrating phylogenetic evidence and genetic divergence metrics, the population from Pengshui Miao and Tujia Autonomous County represents a distinct evolutionary lineage, warranting recognition as a new species within the genus *Paramesotriton*.

Taxonomic account

***Paramesotriton chongqingensis* sp. nov.**

https://zoobank.org/2C6ED903-E27B-4375-9951-88AF0CC49FE6

Type material. ***Holotype*:** CMNH1662, adult male, collected from Ditang Township, Pengshui Miao and Tujia Autonomous County, Chongqing Municipality, China (29.61°N, 108.19°E; 718 m elevation) by Qi Ma on 30 June 2025.

***Paratypes*:** CMNH 1575 (female) collected by Qi Ma,Lipeng Wan on 18 May 2023 at the same locality as the holotype; CMNH 1658, CMNH 1659, CMNH 1660 (males), and CMNH 1661 (female), all collected at the same locality and date as the holotype.

Etymology. The specific epithet "chongqingensis" is named after the type locality, Chongqing, China. We suggest the English common name "Chongqing Warty Newt" and the Chinese common name "重庆瘰螈" (Chóng qìng luǒ yuán).

Diagnosis. The new species can be distinguished from its congeners by the following combination of morphological characteristics: (1) rough skin; (2) coloration of the vertebral ridge similar to, or slightly lighter than, the body coloration; (3) presence of horn-like projections posterodorsally on the head; (4) ventral surface of throat and body black with irregular orange-red or orange-yellow spots or streaks; (5) absence of vestigial gills and gill filaments in adults; (6) tail tip in a bluntly rounded tip; (7) absence of spotting on cheek, lateral sides of dorsum, lateral sides of tail, base of limbs; (8) forelimbs relatively long, when stretched forward along body their fingertips extend beyond the anterior corner of the eye; when forelimbs and hind limbs adpressed, palm and tarsus overlap; (9) both males and females possess yellow-white caudal stripes; ventral orange-red stripe extends from cloaca to tail tip or covers only proximal part near cloaca, disappearing at approximately 1/8 of tail length.

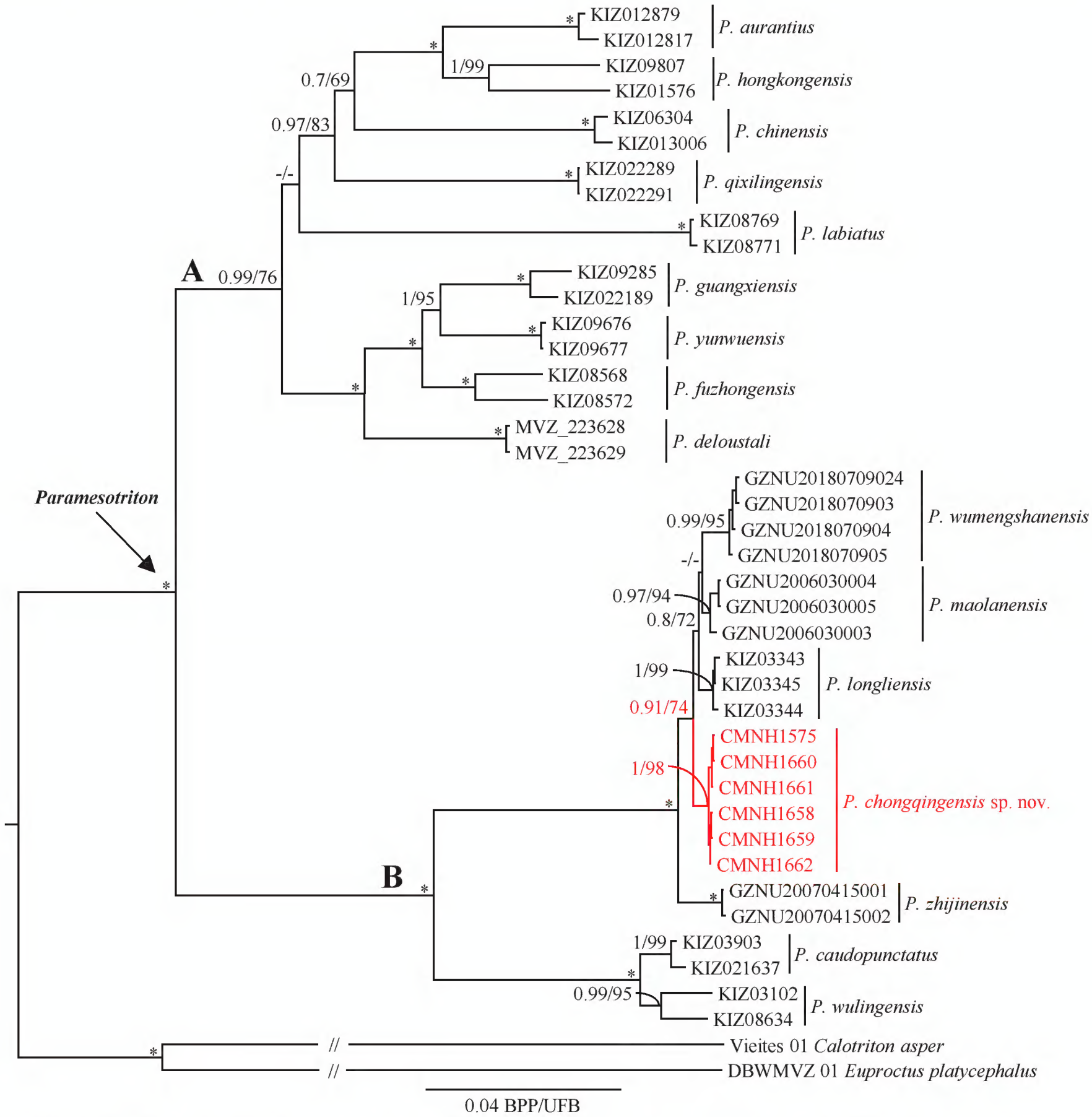

Figure 2. BI tree based on the *ND2*, *Cytb* and *POMC* gene combined sequences. BPP from BI analyses/UFB from ML analyses are listed next to the nodes. Asterisks and "-" indicate support values of 1.00/100 and below 0.60/60. The scale bar represents 0.04 nucleotide substitutions per site. The new samples for the present study are indicated by the red font. This illustration was created and provided by Qi Ma.

Description of the holotype. The adult male specimen is well-preserved. SVL 77.2 mm, TAL 56.5 mm, for further measurements see Table 1. Body relatively slender; head slightly flattened with a horn-like projection present on the posterior dorsal side of the head; head length exceeding head width (HL/HW = 1.31); snout length greater than eye diameter (SL/ED = 2.65); snout truncate in dorsal and lateral views, projecting beyond lower lip; canthus rostralis distinct; nostrils positioned anteriorly on lateral snout margins; rictus extending to lower corner of the eye; labial folds well-developed; upper and lower jaws bearing small teeth; vomerine teeth arranged in a "Λ" shape;tongue elliptical, adhering to mouth floor with anterior and lateral margins not free or slightly free.

Limb lengths subequal; hindlimbs slightly more robust than forelimbs; forelimbs relatively long, finger tips extending beyond anterior corner of eye when adpressed anteriorly along body; palm and tarsus overlapping when forelimbs and hindlimbs adpressed against body; lateral fringes and webbing absent on fingers and toes; four fingers, five toes, relative finger lengths I < IV < II < III; relative toe lengths I < V < II < IV < III; tail length shorter than snout-vent length (TAL/SVL = 0.69), base cylindrical, gradually becoming laterally compressed distally with bluntly rounded tip; skin rough; vertebral ridge prominent, dorsal surfaces of trunk, tail, and limbs bearing scattered tubercles and granular glands of varying sizes; ventrolateral skin rough with small tubercles; cloaca region swollen with transverse wrinkles

Table 1. Measurements (mm) of adult specimens in the type series of *Paramesotriton chongqingensis* sp. nov.

Trait	CMNH 1662*	CMNH 1575	CMNH 1658	CMNH 1659	CMNH 1660	CMNH 1661
SEX	male	female	male	male	male	female
TOL	132.3	107.6	129.2	129.5	138.8	147.3
SVL	77.2	66.7	77.1	71.9	77.7	81.3
HL	21.2	19.6	21.7	21.8	21.8	21.0
HW	16.9	13.7	16.5	16.5	18.5	17.3
TRL	56.6	51.0	57.6	50.8	57.9	61.2
EN	5.3	5.3	6.2	5.2	5.8	5.1
IND	4.5	4.3	4.3	4.1	4.2	4.9
AG	33.4	27.8	34.6	30.8	30.4	39.2
TAL	56.5	38.8	53.2	58.9	58.9	64.7
TAD	11.2	7.6	11.1	11.9	11.5	10.6
TAW	10.8	6.6	10.0	10.5	10.5	9.8
FLL	25.1	18.8	23.8	21.9	26.6	24.1
HLL	22.7	16.5	25.8	22.3	25.4	25.1
CHW	13.5	10.8	13.2	14.5	15.7	14.0
IOD	8.2	5.1	8.7	8.9	9.5	8.5
ED	3.6	3.7	2.9	3.5	4.1	4.0
SL	7.1	6.6	7.7	7.5	8.0	7.4

Color of holotype in life. Dorsal and lateral color of head, body, limbs, and tail tawny yellow. Vertebral ridges concolorous or slightly lighter. Ventral surfaces of gular region and body black with irregular orange-red or orange-yellow spots/streaks. Orange-red blotch anterior to cloaca. Orange-red stripe from cloacal posterior margin along ventral tail surface (proximal 1/8). Distinct yellow-white band laterally from tail base to tip (Fig. 3).

Color of holotype in preservative. Dorsal and lateral coloration of head, trunk, limbs, and tail unchanged or slightly faded; ventral markings show fading: orange-red spots on gular and body venter, orange-red blotch anterior to cloacal opening, and orange-red stripe on ventral tail surface all faded to yellow or cream-yellow (Fig. 4).

Variation. Linear measurements summarized in Table 1. Paratypes morphologically similar to holotype; adult dorsum tawny or dark brown; ventral pattern variable: irregular orange-red spots on venter or connected forming orange-red/orange-yellow streaks; orange-red stripe from cloacal posterior margin confined to proximal tail (terminating at approximately posterior 1/8 or extending to tip); distinct or faint yellow-white tail band present (Fig. 5).

Sexual dimorphism. Male cloaca: swelling large and low; anal opening a longitudinal slit. Female cloaca: swelling small and high; anal opening small, elliptical (Fig. 6).

Ecology and distribution. *Paramesotriton chongqingensis* sp. nov. inhabits montane streams at 700–1000 m elevation; water cold, clear, slow-flowing; substrate with sand, leaf litter, rocks, aquatic plants. Co-occurrence with stream fishes and small aquatic invertebrates. Stream width 5–10 m; mostly shallow except scattered pools (2–3 m deep). Riparian zone densely vegetated (evergreen forest); stream unshaded. Diurnally concealed at bottom or edges of the stream (Fig. 7).

Comparisons. *Paramesotriton chongqingensis* sp. nov. is phylogenetically close to *P. zhijinensis*, *P. maolanensis*, *P. longliensis* and *P. wumengshanensis*, but differs in the following (Figs 8, 9).

Paramesotriton chongqingensis sp. nov. differs from *P. zhijinensis* by lacking vestigial gill or gill filaments on each side behind the head in adults (vs. present); vomerine ridges form a wider angle, not curved (vs. narrower and curved).

P. chongqingensis sp. nov. differs from *P. maolanensis* in having rough skin and normally developed eyes (vs. smooth skin and reduced eyes in *P. maolanensis*); ventral surfaces of the gular region and body black with clear and sparse distinct irregular orange-red or orange-yellow spots or streaks (vs. small dense yellow dots present along ventrolateral body).

Paramesotriton chongqingensis sp. nov. differs from *P. longliensis* in posterior tail region not pale purplish-red in males; both sexes having paired yellowish-white caudal stripes (vs. posterior tail pale purplish-red without markings in male); ventral fin fold on postcloacal tail orange-red, extending to tail tip or fading approximately at posterior one-eighth (vs. orange-red only on posterior half); tongue elliptical, adhering to mouth floor with anterior and lateral margins not free or slightly free (vs. tongue adhering to mouth floor with free lateral margins); ; vomerine ridges forms an wider angle, not curved (vs. narrower and curved); dorsal skin distinctly rough (vs. comparatively smooth).

Paramesotriton chongqingensis sp. nov. differs from *P. wumengshanensis* in ventral surfaces of the gular region and body black with irregular orange-red or orange-yellow spots or streaks (vs. head, body, and ventral surfaces of limbs dark brown overall with irregular reddish-orange markings, which are typically larger); ventral orange-red stripe extends from cloaca to tail tip or covers only proximal part near cloaca, disappearing at approximately 1/8 of tail length (vs. a single red-orange or tangerine yellow stripe extends from the posterior margin of cloaca to the 4/5 parts of the tail on the ventral side) (Fig. 9).

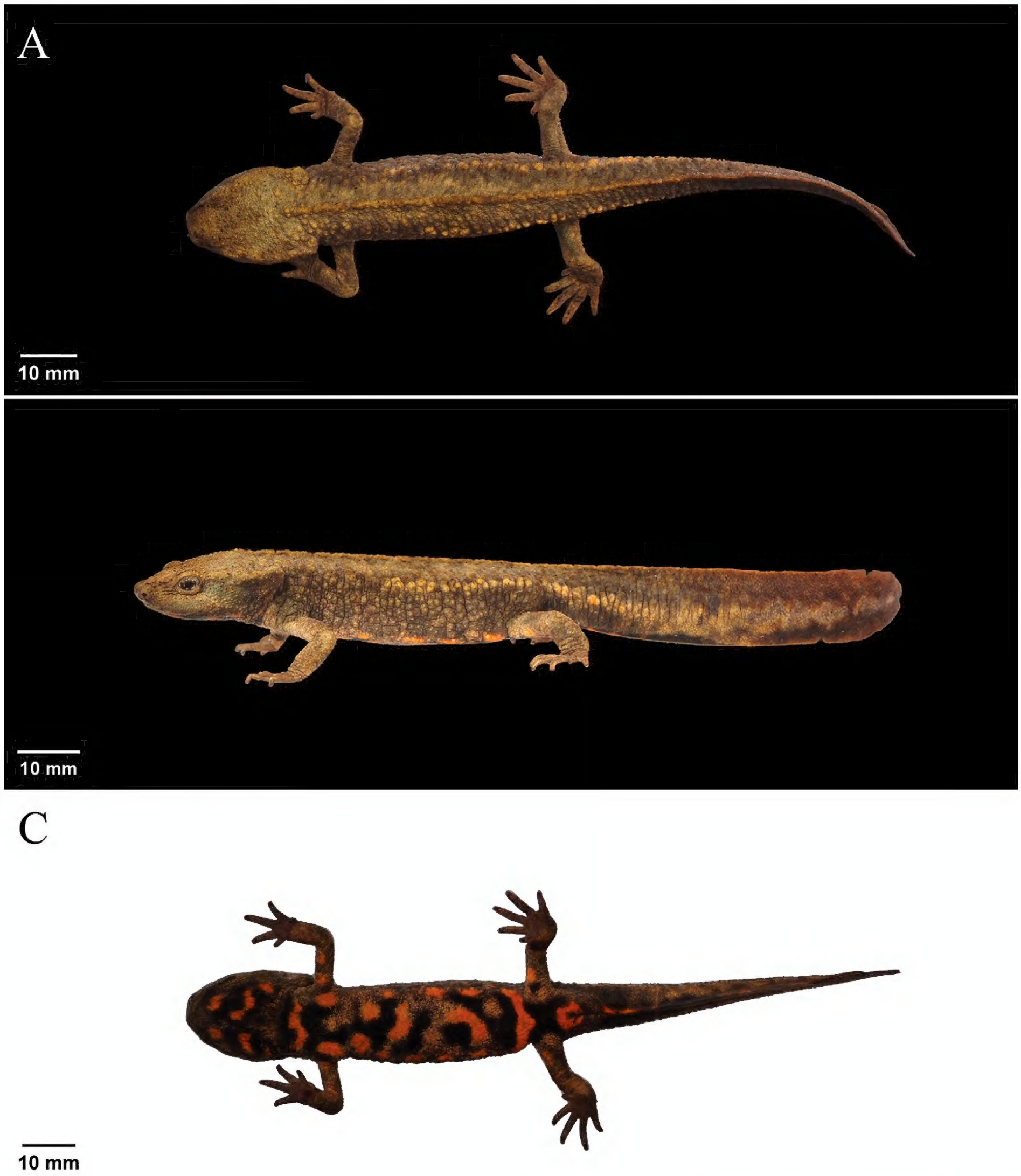

Figure 3. Holotype of *Paramesotriton chongqingensis* sp. nov. (CMNH1662) in life. **A.** Dorsal view; **B.** Lateral view; **C.** Ventral view. Photos by Qi Ma.

Paramesotriton chongqingensis sp. nov. differs from *P. hongkongensis*, *P. labiatus*, and *P. maolanensis* in having rougher skin (vs. skin smooth). It differs from *P. aurantius*, *P. chinensis*, *P. caudopunctatus*, *P. wulingensis*, and *P. hongkongensis* by having the dorsal vertebral ridge coloration closely matching the body color or slightly brighter (vs. bright orange and continuous in *P. aurantius*; brown or maroon in *P. chinensis*; earth-yellow longitudinal stripes on vertebral ridge and dorsolateral ridges in *P. caudopunctatus* and *P. wulingensis*; distinctly light-colored in *P. hongkongensis*). *P. chongqingensis* sp. nov. differs from *P. aurantius*, *P. caudopunctatus*, *P. chinensis*, *P. deloustali*, *P. fuzhongensis*, *P. guangxiensis*, *P. hongkongensis*, *P. labiatus*, *P. malipoensis*, *P. qixilingensis*, *P. wulingensis*, *P. yunwuensis*, and *P. zhijinensis* by possessing paired horn-like projections posterodorsally on the head (vs. absent). It differs from *P. caudopunctatus*, *P. wulingensis*, *P. deloustali*,

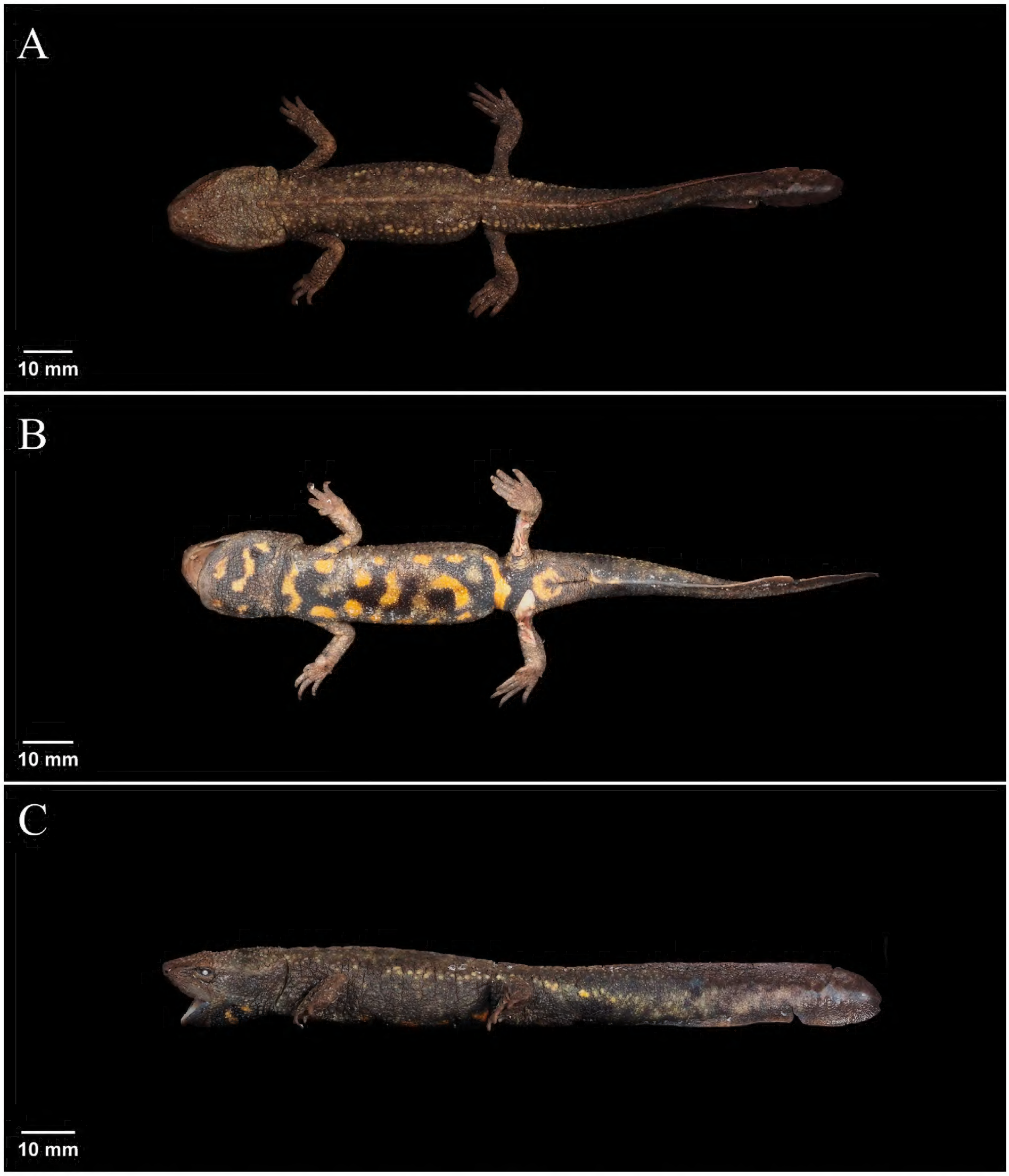

Figure 4. Holotype of male *Paramesotriton chongqingensis* sp. nov. (CMNH1662) in preservation. **A.** Dorsal view; **B.** Ventral view; **C.** Lateral view. Photos by Qi Ma.

P. guangxiensis, and *P. yunwuensis* in having the chin and venter black, mottled with irregular orange-red or orange-yellow blotches or streaks (vs. pale venter with dark blotches in *P. caudopunctatus* and *P. wulingensis*; large orange ventral blotches in *P. deloustali*, *P. guangxiensis*, and *P. yunwuensis*). It differs from *P. qixilingensis* in having a bluntly rounded tail tip (vs. tail tapering distally). *P. chongqingensis* sp. nov. differs from *P. aurantius*, *P. caudopunctatus*, *P. wulingensis*, and *P. malipoensis* by lacking blotches on the cheeks, lateral dorsum, tail sides, and limb bases (vs. irregular yellow spots present in these areas in *P. aurantius*; purple-red spots bordered with black on lateral tail surfaces and pale lower lateral tail surface with small black spots in *P. caudopunctatus* and *P. wulingensis*; distinct small orange-red or yellowish-orange spots dorsolaterally in rows extending to mid-tail in *P. malipoensis*). It differs from *P. yunwuensis* in having finger tips extending beyond the anterior corner of the eye when forelimbs are stretched forward along body (vs. barely reaching posterior eye margin).

Figure 5. Paratype of female *Paramesotriton chongqingensis* sp. nov. (CMNH 1661) in life. **A.** Dorsal view; **B.** Lateral view; **C.** Ventral view. Photos by Qi Ma.

Discussion

Based on morphological and molecular evidence, we describe a new species of Asian warty newt, *Paramesotriton chongqingensis* sp. nov., discovered in montane streams of Pengshui Miao and Tujia Autonomous County, Chongqing Municipality, China. This discovery not only confirms the presence of unexplored cryptic lineages within biodiversity hotspots such as the Wuling Mountains (Luo et al. 2021; Yuan et al. 2022a),but also presents a compelling case for species delimitation in the face of minimal mitochondrial divergence, a topic of ongoing debate in herpetological taxonomy (Wüster 2025).

The new species is assigned to the *P. caudopunctatus* clade (Clade B). Molecular phylogenetic analyses, based on a combined dataset of *ND2*, *Cytb*, and *POMC*, reveal that *P. maolanensis*, *P. longliensis* and *P. wumengshanensis* form a strongly supported group (BPP = 0.8, BS = 72). *Paramesotriton chongqingensis* sp. nov. constitutes a well-supported sister lineage to this clade (BPP = 0.91,

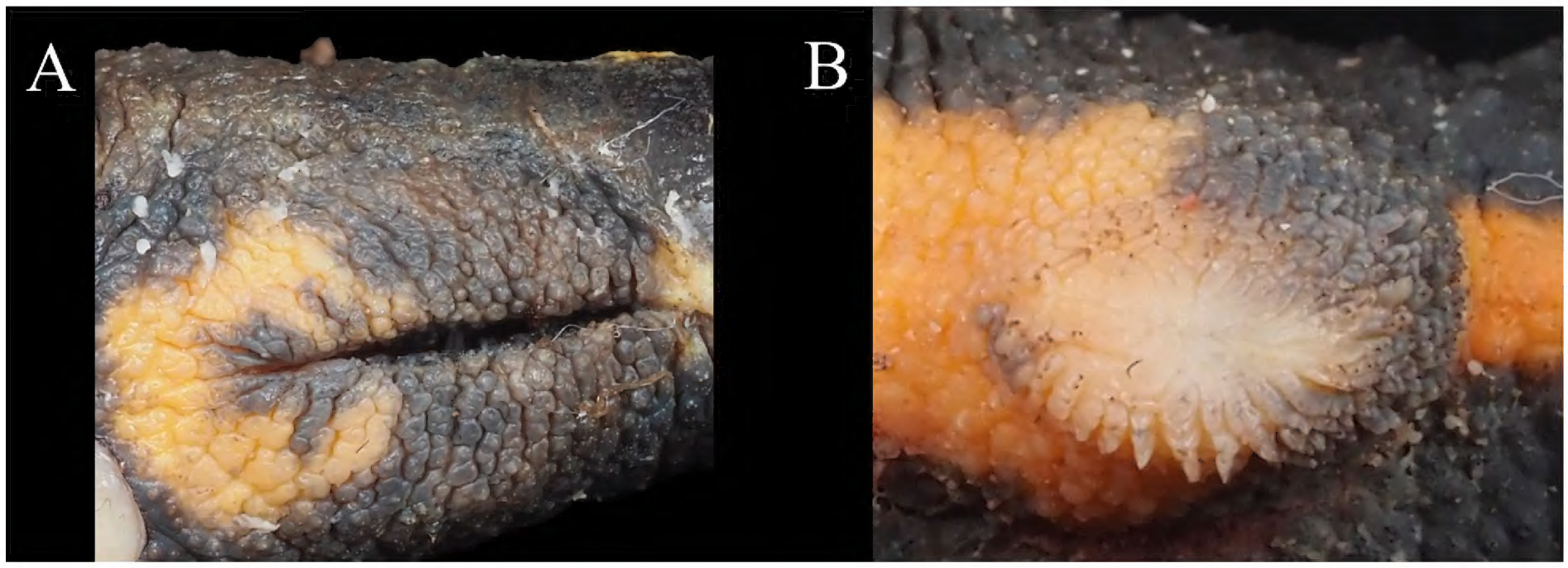

Figure 6. The cloaca of male *Paramesotriton chongqingensis* sp. nov. (**A.** CMNH1662) and female *Paramesotriton chongqingensis* sp. nov. (**B.** CMNH1661) in preservation. Photos by Qi Ma.

Figure 7. Habitat of *Paramesotriton chongqingensis* sp. nov. at the type locality in Ditang Township, Pengshui Miao and Tujia Autonomous County, Chongqing, China. **A.** Habit; **B.** Ecological photo of specimen CMNH1575. Photos by Qi Ma.

BS = 74). Intraspecific genetic distance within the new species is zero, while its divergence from its closest relative, *P. maolanensis*, reaches 0.78% (*Cytb*, p-distances). This genetic distance significantly exceeds the intraspecific variation observed in some congeners (e.g., 0.16% in *P. longliensis*) and is greater than the interspecific distance between *P. maolanensis* and *P. longliensis* (0.63%).

However, as critically discussed by Wüster (2025), mitochondrial DNA (mtDNA) alone is an insufficient marker for species delimitation due to its propensity for cytonuclear discordance, historical introgression, and its inability to reflect biparental gene flow. Relying solely on mtDNA thresholds can lead both to oversplitting of structured populations and to the lumping of independent evolutionary lineages that have experienced mitochondrial capture. Therefore, the critical test for species status lies not in the magnitude of mtDNA divergence, but in the integration of multiple, independent lines of evidence that collectively demonstrate evolutionary independence. In this context, the minor mitochondrial differences observed within the species group containing *Paramesotriton chongqingensis* sp. nov., *P. longliensis*, *P. maolanensis*, *P. wumengshanensis* and *P. zhijinensis* do not negate their specific status; rather, they highlight the necessity of our integrative approach. According to robust morphological comparison (including vomerine tooth morphology, structure of hyoid apparatus and statistical analysis), phylogenetic analysis based on ND2, mitogenome, and 1,475,246

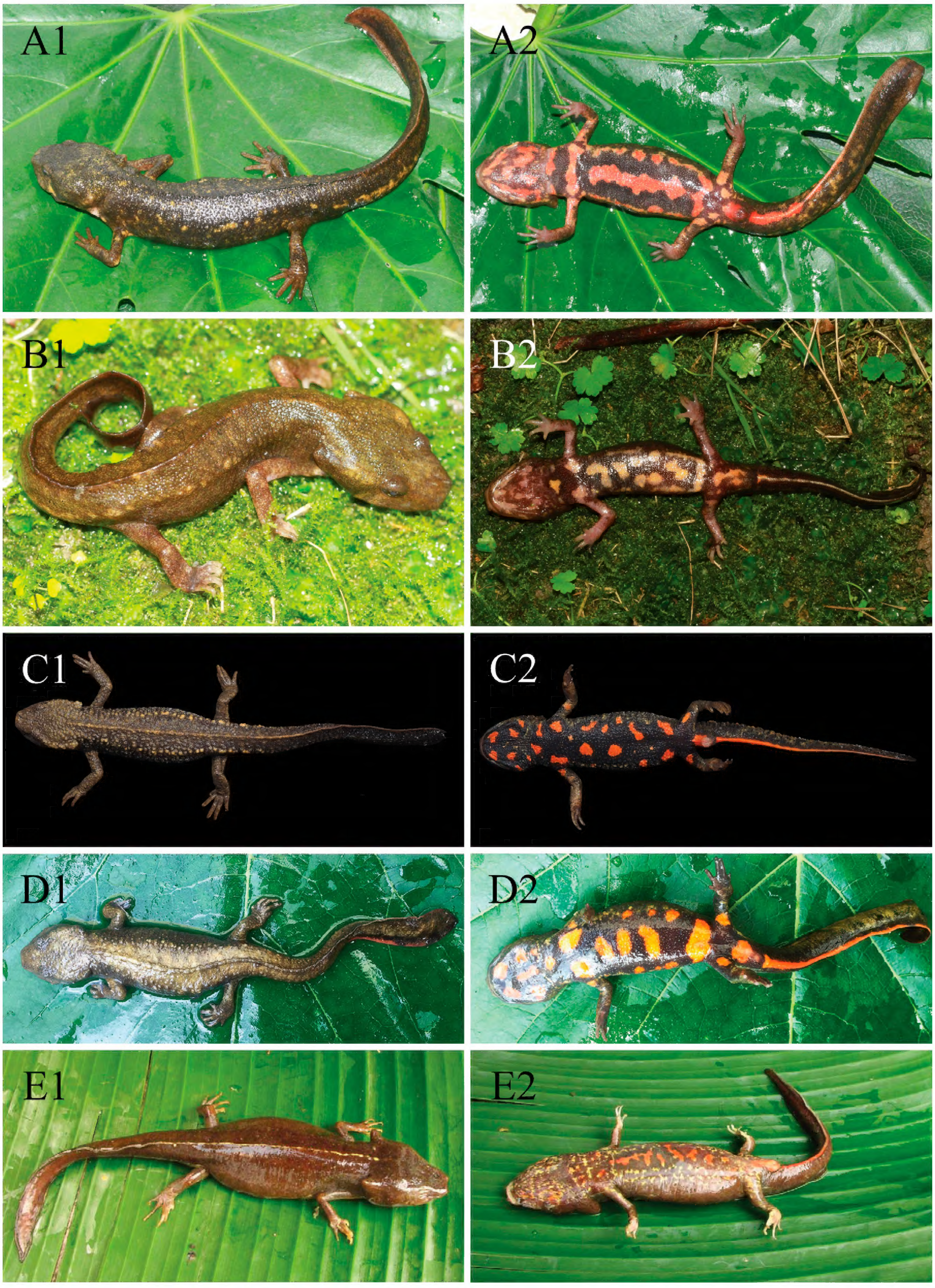

Figure 8. Dorsal and ventral views of *Paramesotriton chongqingensis* sp. nov. and four phylogenetically closely related species. **A.** *P. longlisis*; **B.** *P. zhijinensis*; **C.** *P. chongqingensis* sp. nov.; **D.** *P. wumengshanensis* (modified from Luo et al. 2025).; **E.** *P. maolanensis* (modified from Gu et al. 2012a).

nuclear single nucleotide polymorphisms (SNPs), the species in this group exhibits diagnostic characters distinguishing from congeners and their mitochondrial and nuclear data consistently resolve as distinct lineage (Luo et al. 2025). The minor mitochondrial divergence in few mitochondrial DNA of the species group could be a result of historical gene flow and/or incomplete lineage sorting according to Luo et al. (2025). Another case from Southwestern China is that mitogenome introgression between *Odorrana grahami* complex in southern China led to

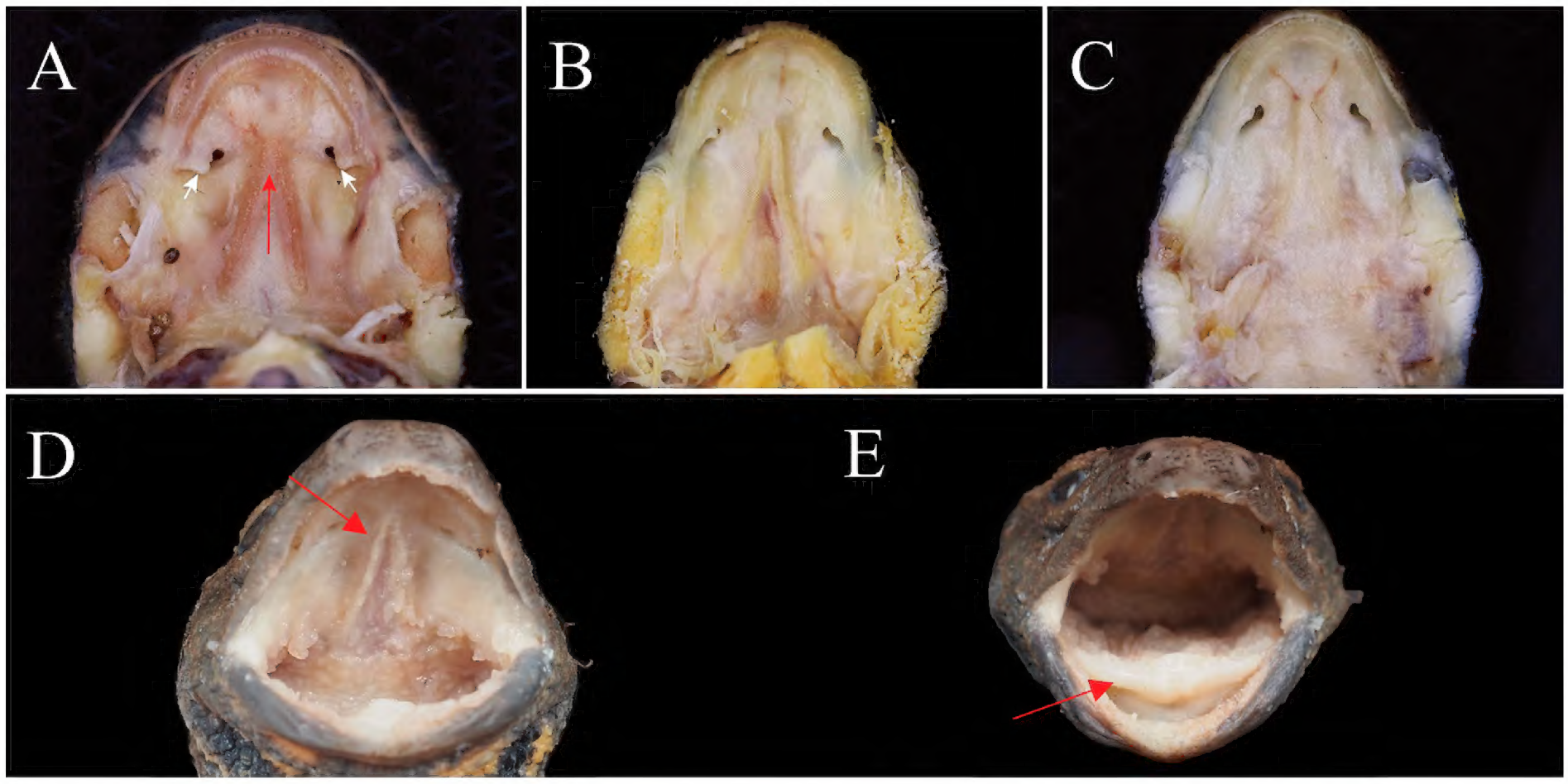

Figure 9. Comparative morphology of the vomerine teeth in *Paramesotriton chongqingensis* sp. nov. and three other congeners within the *P. caudopunctatus* group. **A.** *P. wumengshanensis*. The white arrow indicates the bony plate posterior to the choana; the red arrow points to the groove anterior to the vomerine tooth; **B.** *P. zhijinensis*; **C.** *P. longliensis*; **D.** *P. chongqingensis* sp. nov. The red arrow indicates the vomerine teeth; **E.** Oral cavity of *P. chongqingensis* sp. nov. The red arrow indicates the tongue. Images **A–C.** are modified from Luo et al. (2025).

minimal differences in mitochondrial cytochrome oxidase subunit I (ranging from 0.3% to 0.8%) but significant nuclear gene divergence (Yuan et al. 2022b).

Notably, such relatively small genetic divergences are not uncommon in rapidly diversifying lineages and often reflect recent speciation events, as observed across various amphibian groups. For instance, within the toad genus *Oreolalax* (Megophryidae), multiple species pairs exhibit minimal genetic distances. Specifically, interspecific mitochondrial 16S rRNA genetic distances can be remarkably low (e.g., 0.6% between *O. major* and *O. xiangchengensis*; 0.4% between *O. schmidti* and *O. pingii*), yet these taxa display pronounced morphological differentiation sufficient to justify their recognition as distinct species (Hou et al. 2020).

This phenomenon illustrates a key taxonomic principle: even with limited genetic distance, pronounced and consistent morphological differentiation holds significant diagnostic value in species delimitation. Therefore, comprehensive morphological analysis provides crucial complementary evidence to molecular data, particularly in recently radiated lineages (where genetic drift may not yet fully reflect evolutionary independence). In such cases, morphological characters thus remain the fundamental basis for reliable species identification and lineage recognition (Dufresnes et al. 2021; Hou et al. 2024).

Morphologically, the new species differs from congeners by a suite of stable diagnostic characters: the presence of horn-like projections posterodorsally on the head (present only in *P. maolanensis* and *P. longliensis*); irregular orange-red spots or streaks on a black background on the throat and venter (distinct from the large orange patches of *P. deloustali* and the dark markings on a pale background in *P. caudopunctatus*); a bluntly rounded tail tip (differs from *P. qixilingensis*, which exhibits tail tapering from base to tip); absence of gill vestiges (present in *P. zhijinensis*); and absence of lateral spotting (differs from the yellow spots of *P. aurantius* and the orange-red spots of *P. malipoensis*). This combination of features provides a reliable basis for morphological identification.

From a biogeographic perspective, the discovery site of the new species lies within the Wuling Mountains, a significant hotspot for the evolutionary diversification of *Paramesotriton*, particularly within the *P. caudopunctatus* species group. This species is currently known only from specific stream habitats in Pengshui and exhibits clear geographic isolation from other members of the species group (e.g., *P. maolanensis*, *P. wumengshanensis*, *P. zhijinensis*, *P. longliensis*). This distribution pattern strongly suggests that habitat fragmentation driven by karst topography (allopatric isolation) may be a key mechanism driving speciation within this group, further supporting the hypothesis by Luo et al. (2021, 2022) that the complex geological history and heterogeneous habitats of southwestern China have collectively promoted *Paramesotriton* diversification.

This newly discovered species also faces significant conservation challenges. Its highly specialized habitat requirements (e.g., clear, slow-flowing streams with forest canopy cover) render it highly vulnerable to threats from habitat fragmentation caused by hydropower development and tourism infrastructure. Furthermore, its brightly patterned venter makes it a potential target for the illegal pet trade (Sparreboom 2014). Based on multiple field surveys conducted in the surrounding regions, combined with a review of existing literature (Wang et al. 2013), we have only identified a single known occurrence locality for this species. This limited distribution pattern suggests it may be a point endemic species.

This aligns with IUCN criteria for threatened species. Immediate actions are recommended, including population size assessments, delineation of distribution boundaries, and the inclusion of its habitat within priority conservation networks to mitigate imminent threats to its survival.

Acknowledgments

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Supplementary material 1

Localities and voucher data for all specimens used in this study

Authors: Qi Ma, Shi-Ze Li, Xiao Yang, Li-Peng Wan, Cheng-Cheng Ma, Sheng-Chao Shi, Zhi-Jian Wang

Data type: xlsx

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Link: https://doi.org/10.3897/zse.101.168621.suppl1

Supplementary material 2

Average uncorrected p-distances (percentage) among *Paramesotriton* individuals calculated from *Cytb* gene sequences (below the diagonal) and standard error estimates (above the diagonal)

Authors: Qi Ma, Shi-Ze Li, Xiao Yang, Li-Peng Wan, Cheng-Cheng Ma, Sheng-Chao Shi, Zhi-Jian Wang

Data type: xlsx

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